PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Applicant's or agent's file reference see form PCT/ISA/220	Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet) **FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/008470	29.07.2004	12.08.2003

International Patent Classification (IPC) or both national classification and IPC
C12P13/08, C12N15/00

Applicant
DEGUSSA AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/008470

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

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Box No. II Priority

1. ☐ The following document has not been furnished:

   ☐ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-34, 36-39
	No:	Claims	35
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-35
Industrial applicability (IA)	Yes:	Claims	1-35
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

see form 210

Re Item IV

This Authority considers that there are 2 inventions covered by the claims indicated as follows:

I: Claims 1-34 and 36-39 directed to a process for the preparation of L-threonine using L-threonine-producing bacteria from the family Enterobacteriaceae, as claimed in claim 1.

II: Claim 35 directed to saccharose-utilizing transconjugants of Escherichia coli K-12 deposited as DSM 16293 at the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany)

The reasons for which the inventions are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT, are that the claimed subject-matter does not seem to be linked by a common technical feature defining a contribution over the prior art.

Also, examining the possible correspondence by technical effect, one finds that the technical effect of the first invention is the production of L-Threonine
and that the technical effect of the second invention is the utilization of saccharose by the strain itself.

The application, hence does not meet the requirements of unity of invention as defined in Rules 13.1 and 13.2 PCT.

Since Applicant has payed an additional fee, both groups of inventions are examined here.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:
   D1: US 2002/055151 A1 (RIEPING MECHTHILD ET AL) 9 May 2002 (2002-05-09)
   D2: NAGANO T ET AL: "HIGH EXPRESSION OF THE SECOND LYSINE DECARBOXYLASE GENE, IDC, IN ESCHERICHIA COLI WC196 DUE TO THE RECOGNITION OF THE STOP CODON (TAG), AT A POSITION WHICH CORRESPONDS TO THE 33TH AMINO ACID RESIDUE OF PHI38, AS A

SERINE RESIDUE BY THE AMBER SUPPRESSOR," BIOSCIENCE BIOTECHNOLOGY BIOCHEMISTRY, JAPAN SOC. FOR BIOSCIENCE, BIOTECHNOLOGY AND AGROCHEM. TOKYO, JP, vol. 64, no. 9, 2000, pages 2012-2017, XP009012266 ISSN: 0916-8451

D3: SHIIO I ET AL: "MICROBIAL PRODUCTION OF L-THREONINE PART I. PRODUCTION BY ESCHERICHIA COLI MUTANT RESISTANT TO ALPHA-AMINO-BETA-HYDROXYVALERIC ACID" AGRICULTURAL AND BIOLOGICAL CHEMISTRY, JAPAN SOCIETY FOR BIOSCIENCE, BIOTECHNOLOGY AND AGROCHEMISTRY,, JP, vol. 33, no. 8, 1969, pages 1152-1160, XP001131907 ISSN: 0002-1369

D4: US-A-4 278 765 (DEBABOV VLADIMIR G ET AL) 14 July 1981 (1981-07-14)

D5: WO 03/074719 A (DEGUSSA) 12 September 2003 (2003-09-12)

D6: SMITH H W ET AL: "TRANSMISSIBLE SUBSTRATE-UTILIZING ABILITY IN ENTEROBACTERIA" JOURNAL OF GENERAL MICROBIOLOGY, SOCIETY FOR MICROBIOLOGY, READING, GB, vol. 87, no. 1, 1975, pages 129-140, XP001024294 ISSN: 0022-1287

2. INVENTION 1: process for the preparation of L-threonine using L-threonine-producing bacteria from the family Enterobacteriaceae, as claimed in claim 1.

2.1 Inventive activity (Art 33(3) PCT).

The document D1 (US20020055151) is regarded as being the closest prior art to the subject-matter of claim 1 since it discloses a process for the preparation of L-threonine, using Enterobacteriaceae and wherein some of the fermentation broth is abstracted and up to 90% of the total volume of the fermentation broth remains in the fermentation container, the remaining fermentation broth is topped up with fresh medium, the last two steps are optionally repeated. In addition, the concentration of the sources of carbon is deemed to be below 30 g/l during the cultivation after topping up with new medium (see examples).

It follows that the difference between the teaching of this document and the present application is that, in the present application, more than 90% of the total volume of the fermentation broth remains in the fermentation broth. The problem to be solved by the present invention may therefore be regarded as an improvement of the fermentation

process for the production of Threonine by Enterobacteriaceae. In the absence of comparative data showing that the presently claimed process leads to a surprising effect or a particular advantage when compared to the existing processes for the production of L-Threonine, the present application fails to show that the claimed subject-matter indeed solves the technical problem. The solution proposed in claim 1 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT).

The subject-matter of the dependent claims 2-34 and 36 to 39 are deemed to be obvious embodiments of claim 1.
In particular, the use of thrA and rpoS mutants and other additional mutations was already known from prior art (see e.g. D2-D4 and the literature cited in the present application at pages 24-29). The other embodiments which concern various fermentation conditions seem to be trivial.

At the present time claims 1-34 and 36 to 39 are not inventive over the teaching of prior art.

3. INVENTION 2: saccharose-utilizing transconjugants of Escherichia coli K-12 deposited as DSM 16293 at the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany)

3.1 Novelty and inventive activity (Art. 33(2) and (3) PCT)
D6 discloses the generation of a saccharose-utilizing E. coli K-12 by transconjugation to a nalidixic acid resistant K-12 E. coli strain.

The presently claimed strain, if not identical (and thus not novel) to the published strain, represents an obvious alternative to the published strain and thus, does not show any inventive activity.

Claim 35 does not seem to be novel, and in any case is not inventive over D6.

4. Industrial applicability Art. 33(4) PCT.
The present set of claims shows industrial applicability.

5. Additional comments.
Applicant's attention is drawn to the fact claims 27-34 do not meet the requirements of Art. 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem instead of defining the subject-matter in terms of technical features.

Claim 39 neither complies with Art. 6 PCT since it embraces so many embodiments that it is not concise.

Re Item VI

Certain documents cited

The present opinion was drafted as if the priority was valid. should this not be the case, document D5 will become relevant for assessing novelty and inventive activity.